Analysis of Cocaine

1. Background

Cocaine is produced from alkaloids obtained from the plant *Erythroxylon coca*. Cocaine, as the hydrochloride salt, is a white or off white powder known as snow. This salt may be re-converted to the freebase form, which is more volatile and produces hard, waxy lumps known as rocks or crack. The cocaine molecule contains two chiral carbon atoms making it possible for four different isomeric forms. However, l-cocaine is the only enantiomer which occurs naturally and some courts have ruled it alone is an illegal drug. This differs from synthetic cocaine which can contain a racemic mixture of all four isomeric forms. Cocaine is ingested as the hydrochloric salt by either insufflation or intravenous injection. As a freebase, not only could cocaine be smoked, but it is more fat-soluble, which allows it to cross into the brain quicker. Since cocaine can be ingested by several different routes, a variety of paraphernalia can be associated with cocaine samples. For cocaine salt, these items might include plastic bags, spoons, syringes, razor blades, straws, etc. For cocaine base, these items might include plastic bags, pipes or scouring pads. Samples will be analyzed by physical description, weight (if applicable), preliminary tests and then followed by confirmatory test.

2. Objective

The objective of this SOP is to establish guidelines to be used for the analysis of a sample that may contain cocaine, specifically l-cocaine.

3. Scope

This SOP is to be used by the laboratory staff of the Division of Analytical Chemistry at William A. Hinton State Laboratory Institute in Boston, MA.

4. Responsibility

Chemists are responsible for acquiring glassware, preparing chemical reagents and standards, sample analysis, and reporting. Chemists also perform instrument calibrations, maintenance and troubleshooting, ordering of supplies and other necessary tasks related to this analysis.

Technical Reviewers will review each case and complete the comprehensive reviewer checklist. They will ensure that the chemist followed this SOP. The Technical Reviewer may perform the duties and responsibilities of the chemist.

Laboratory Supervisors ensure that chemists are following this SOP. They may perform the duties of the chemists and must review raw data and reports generated by chemists. The Supervisor may advise the chemists of alternative testing methods. They ensure that quality control measures are within acceptable limits and determine when corrective actions are needed. They coordinate proficiency testing (PT), reporting and distribution of PT results. They oversee sample results distribution to outside agencies.

Directors ensure that the SOP is being followed and reviewed on a regular basis. They provide approval of standard operating procedures and review quality control documentations.

1. Related Documents

Cole, Michael, "The Analysis of Controlled Substances," London: John Wiley & Sons Ltd., 2003
Drug Enforcement Administration, "Basic Training Program for Forensic Drug Chemists," Drug Enforcement Administration.
Mills III, Terry et al, "Instrumental Data for Drug Analysis," 3rd ed., 6 vols., New York: CRC Press, 2006.
Moffat, A.C. et al, "Clarke's Isolation and Identification of Drugs," 2nd ed., London: The Pharmaceutical Press, 1986.
Moffat, A.C. et al. "Clarke's Analysis of Drugs and Poisons," 3rd ed., London: The Pharmaceutical Press, 2004.
Saferstein, Richard, "Forensic Science Handbook," New Jersey: Prentice Hall, 1988.
Scientific Working Group for the Analysis of Seized Drug Recommendation, 6th ed., "Part III A & B, Methods of Analysis/Sampling of Seized Drug for Qualitative Analysis," July 2011

6. Definitions

AuCl: Gold Chloride
FTIR: Fourier Transform Infrared Spectroscopy
GC w/ FID: Gas Chromatography with Flame Ionization Detector
GC/MS: Gas Chromatography/Mass Spectrometry
Gross Weight: The weight of both the substance and its container.
Net Weight: The weight of the substance only.
TLTA: +/-D-pi-tolouyl-d-tartaric Acid
TPE: Tetraphenylethylene

{ DATE \@ "M/d/yyyy" }

7. Supplies, Equipment & Reagents

Supplies

GC columns (30m x 0.32mm x 0.25um)
- HP-1 (Agilent, Cat # 19091Z-413 or equivalent)
- HP-5 (Agilent, Cat # 19091J-413 or equivalent)

GC crimp vials
- Clear (Agilent, 2mL, Cat # 5182-0543 or equivalent)
- Amber (Agilent, 2mL, Cat # 5181-3376 or equivalent)
- Clear, residue (Agilent, 0.3mL, Cat# 9301-0977 or equivalent)

GC/MS columns (30m x 0.25mm x 0.25um)
- HP-1MS (Agilent, Cat # 19091S-933 or equivalent)
- HP-5MS (Agilent, Cat # 19091S-433 or equivalent)

Kimwipes
Pasteur pipette
Porcelain spot plate
Scissors
Spatula
Stirring rod
Teflon crimp (top) caps
- Silver (Agilent, Cat # 5181-1210 or equivalent)
- Blue (Agilent, Cat # 5181-1215 or equivalent)
- Green (Agilent, Cat # 5181-1216 or equivalent)

Various Class A glassware
- Beakers (range 10mL to 1000mL)
- Graduated cylinders (range 25mL to 100mL)
- Volumetric flask (range 10mL to 50mL)

Weighing dish (VWR, Anti-Static, Cat # 89106 or equivalent)
Weighing paper (VWR, Cat # 12578 or equivalent)

Equipment

Analytical Balance (range 0.0001g to 32,000g)
FTIR (Perkin Elmer, Model # Spectrum One or equivalent)
GC with FID (Agilent, Model # 6890 Series or equivalent)
GC/MS (Agilent, Model # 5975 Series or equivalent)
Microscope, Micromaster (Fisher Scientific, Cat # 12-561 or equivalent)
Stereomicroscope, Stereomaster (Fisher Scientific, Cat # 12-562 or equivalent)

Reagents

+/-D-pi-tolouyl-d-tartaruc acid, monohydrate, 98% (Aldrich, Cat # 108081 or equivalent)
Acetone (JT Baker, Ultra Resi-Anhydrous, Cat # 9254 or equivalent)
Alcohol, anhydrous (JT Baker, ACS Grade, Cat # 9401 or equivalent)
Chloroform (JT Baker, ACS Grade, Cat # 9180 or equivalent)
Cobalt thiocyanate (Aldrich, Cat # 216135 or equivalent)
Cobaltous acetate tetrahydrate (Fisher Scientific, Certified, Cat # C364 or equivalent)
Deionized water (in-house)
Formaldehyde, 37% wt solution (Acros Organic, ACS Grade, Cat # AC41073 or equivalent)

Glacial acetic acid (JT Baker, ACS Grade, Cat# 9511 or equivalent)
Glycerol, anhydrous (JT Baker, ACS Grade, Cat # 2136 or equivalent)
Gold Chloride (Fisher Scientific, ACS Grade, Cat # G-54 or equivalent)
Hydrochloric acid, 36.5-38.0% (JT Baker, ACS Grade, Cat # 9535 or equivalent)
Isoproplyamine, 99% (Acros Organic, Cat # AC14892 or equivalent)
Methanol (JT Baker, ACS Grade, Cat # 9070 or equivalent)
Selenous acid, 99.999% (Acros Organic, Cat # 43712 or equivalent)
Sodium molybdate (JT Baker, ACS Grade, Cat # 3764 or equivalent)
Sulfuric acid (JT Baker, ACS Grade, Cat # 9681 or equivalent)

Standards

Cocaine hydrochloride (USP, Cat # 14300 or equivalent)
Codeine phosphate (Grace/Alltech, Cat # 01801 or equivalent)
Tetraphenylethylene, 98% (Aldrich, Cat # T26204 or equivalent)

8. Safety

Due to the potential hazards, appropriate precautions should be taken as necessary. This includes, but is not limited to, the use of fume hoods, gloves, masks and safety glasses. Lab coats are to be worn at all times in the unit, unless performing administrative duties.

9. Reagent/Standard Preparation

Cobalt Thiocyanate Reagent
Dissolve 2.0g of cobalt thiocyanate in 100mL of deionized water. Mix the solution until completely dissolved.

Marquis Reagent
Dilute 10mL of 37% formaldehyde solution in 90mL of concentrated sulfuric acid. While stirring, slowly add the concentrated sulfuric acid to the formaldehyde solution. Allow the solution to cool completely.

Froehde's Reagent
Dissolve 0.5g of sodium molybdate in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

Mecke's Reagent
Dissolve 1.0g of selenous acid in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

2.8N Hydrochloric Acid Reagent
Dilute 92.6mL of 12.1N hydrochloric acid in 400mL of deionized water. Mix the solution completely.

20% Acetic Acid Reagent
Dilute 100mL of glacial acetic acid in 400mL of deionized water. Mix the solution completely.

+/-D-pi-tolouyl-d-tartaric Acid Reagent

Dissolve 100mg of TLTA in 10mL of anhydrous alcohol in a 100mL graduated cylinder. Bring to a 99mL volume with deionized water and then add 1mL of anhydrous glycerol to the solution. Mix the solution completely.

25% Hydrochloric Acid
Dilute 25mL of hydrochloric acid into a 100mL graduated cylinder and bring to volume with deionized water. Mix the solution completely.

Gold Chloride
Dissolve 5g of gold chloride with 25% hydrochloric acid and bring to a 99mL volume in a 100mL graduated cylinder. Add 1mL of anhydrous glycerol to the solution. Mix the solution completely.

Cocaine/Codeine Standard (QC Mix)
Dissolve 10.0 mg of cocaine hydrochloride and 10.0mg of codeine phosphate and bring to volume with 10mL of methanol. Mix the solution until completely dissolved.

Cocaine Standard
Dissolve 10.0mg of cocaine hydrochloride in 10mL of methanol. Mix the solution until completely dissolved.

Tetraphenylethylene (TPE) Internal Stock Solution [20mg/mL]
Dissolve 1.0g of tetraphenylethylene in chloroform and bring to volume using a 50mL volumetric flask. Mix the solution until completely dissolved.

Cocaine Hydrochloride Stock Solution [2mg/mL]
Dissolve 1mg of cocaine hydrochloride in chloroform and bring to volume using a 50mL volumetric flask.

Cocaine Hydrochloride Quantitation Standards

Concentration of 1.0mg/mL of Cocaine Hydrochloride [1.0mg/mL]
Dilute 25mL of cocaine hydrochloride stock solution and add 2mL of TPE stock solution in a 50mL volumetric flask. Bring to volume with chloroform.

Concentration of 0.80mg/mL of Cocaine Hydrochloride [0.80mg/mL]
Dilute 10mL of cocaine hydrochloride stock solution and add 1mL of TPE stock solution in a 25mL volumetric flask. Bring to volume with chloroform.

Concentration of 0.40mg/mL of Cocaine Hydrochloride [0.40mg/mL]
Dilute 5mL of cocaine hydrochloride stock solution and add 1mL of TPE stock solution in a 25mL volumetric flask. Bring to volume with chloroform.

Concentration of 0.24mg/mL of Cocaine Hydrochloride [0.24mg/mL]
Dilute 3mL of cocaine hydrochloride stock solution and add 1mL of TPE stock solution in a 25mL volumetric flask. Bring to volume with chloroform.

10. Procedure

A. Sample Analysis

i. Once the chemist has taken custody of the sample, the sample will be brought to the chemist work area and stored in a secure manner at all times.
ii. The chemist will analyze one lab number at a given time.
iii. Upon analysis of each sample, the chemist will document all observations on the Drug Analysis Form.
iv. They will document the lab number, submitting agency and the evidence gross weight.
v. Once the sample is opened, the chemist will record its physical description, including color, consistency and ballistics, the number of samples and packaging material.
vi. They will obtain a gross weight and the net weight of the sample/s, if applicable.
vii. Then the chemist will proceed to analyze the unknown substance by performing preliminary and confirmatory tests.

B. Sampling Plan

i. If there are less than 10 packages, only one package will be samples and analyzed.
ii. If there are 11 to N packages, randomly select a number of packages equal to 10% of the total number of packages rounded to the next highest integer.
iii. If the sample is approaching a weight cut off, then the statistical hypergeometric sampling plan will be used for analysis.
iv. If there are any issues or questions regarding sampling technique, consult the Laboratory Supervisor.

C. Residues

i. Attempt to scrape or remove sample from the device and place onto weighing paper or dish. Or rinse the device containing the sample with 1-2ml of the chloroform and place the extract into a labeled beaker.
ii. Transfer some of the sample or extract into a labeled residue vial for GC and GC/MS analysis. Residual samples should be dissolved or diluted in chloroform. Cap and seal the vial tightly.
iii. Use the remaining sample or extract to perform the color test.

D. Preliminary Test

i. Color Test

a. The color test consists of four reagents, which are cobalt thiocyanate, marquis, froehde's, and mecke's.
b. For powdered substances, place a couple of drops of cobalt thiocyanate, marquis, froehde's, mecke's reagents into four individual wells on a porcelain spot plate. Then add a small amount of sample (1-2mg of powder) to each well. Note any color change or reaction.
c. For liquid substances, add a small amount of sample (1-2 drops) into four individual wells on a porcelain spot plate and allow the sample to dry completely. Then place a couple of drops of cobalt thiocyanate, marquis, froehde's, mecke's reagents into each well. Note any color change or reaction.
d. If there is no reaction or no color change with the cobalt thiocyanate, then add 1-2 drops of 2.8N hydrochloric acid to the sample. Note any color change or reaction.
e. The results will be recorded on the Drug Analysis Form by documenting the actual color/s observed. Negative observations will be recorded by stating no reaction or no color change

Interpretation:

a. Cobalt thiocyanate reagent: Formation of a blue color indicates the possible presence of cocaine hydrochloride.
b. If the addition of the cobalt thiocyanate results in no color formation or a weak blue color, then with the addition of 2.8N hydrochloric acid, a blue color develops. This indicates the possible presence of cocaine base.

ii. Microcrystalline Test

a. Gold Chloride

i. For powdered substance, place a small amount of sample (1-2mg) onto a clean microscope slide. For liquid substances, place a small amount of sample (1-2 drops) onto a clean microscope slide and allow the sample to dry completely.
ii. Add a drop of gold chloride and then 25% hydrochloric acid to the sample.
iii. Observe the sample under a polarized light microscope with either 4x or 10x magnification. Note any crystalline formation or reaction.
iv. The results will be recorded on the Drug Analysis Form by documenting the actual crystals observed. Negative observations will be recorded by stating no reaction present.

b. +/- Di-p-tolouyl-D-tartaric Acid

i. For powdered substance, place a small amount of sample (1-2mg) onto a clean microscope slide. For liquid substances, place a small amount of sample (1-2 drops) onto a clean microscope slide and allow the sample to dry completely.
ii. Add a drop of di-p-tolouyl-d-tartaric acid to the sample.
iii. Observe the sample under a polarized light microscope with either 4x or 10x magnification. Note any crystalline formation reaction.
iv. If there is no reaction with the TLTA, then add 1-2 drops of 20% acetic acid to the sample. Note any crystalline formation reaction.
v. The results will be recorded on the Drug Analysis Form by documenting the actual crystals observed. Negative observations will be recorded by stating no reaction.

Interpretation

i. Gold Chloride: Formation of X-shaped crystals indicate the presence of cocaine.
ii. TLTA: Once cocaine presence is determined by the gold chloride test, then the sample will be verified for the presence of the L isomer. The formation of a multitude of single needles, tufts, fan-shaped or sheaves crystals indicate the presence of l-cocaine.
iii. If the addition of the TLTA results in no needle formation, and then with the addition of dilute acetic acid, the needles develop. This indicates the possible presence of cocaine base.

iii. Gas Chromatography (as necessary)

a. Place 1-2mg of powder into a labeled GC vial and then add 1.8mL of methanol. Or use the prepared GC vial from section (C).
b. Initiate autosampler sequence using the ROUTINE method running a blank solvent between each unknown sample and Cocaine standard.
c. Compare retention time of the each sample with the reference standard/s. Also check the chromatograph to determine if the sample needs to be diluted or concentrated.
d. Positive GC analysis will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when the retention time of the sample and the

reference standard meet the laboratory criteria and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

Criteria for Gas Chromatography

a. Retention time of the sample must be within +/- 1.5% of the reference standard.
b. The concentration of the sample should be equivalent to the standard. If the sample is too weak, document results on the Drug Analysis Form.

E. Confirmatory Test

i. Gas Chromatography/Mass Spectrometry

a. Confirmatory analysis can be performed using the GC vial from the previous section (D, iii). Or place 1-2mg of powder into a labeled GC vial and then add 1.8mL of methanol.
b. Initiate autosampler sequence using the DRUGS method running a blank solvent between each unknown sample and Cocaine standard.
c. Compare retention time and ion spectra of the each sample with the reference standard.
d. Document the date analyzed and results of the GC/MS onto the MS Tracking Sheet, Drug Analysis Form and Control Card.

Criteria for Gas Chromatography/Mass Spectrometry

a. Retention time of the sample must be within +/- 1.5% of the reference standard.
b. Library spectra match must be > 90%.
c. There must be a visual spectral match between the reference standard and the sample.
d. At least 5 of the major ions must be present for the sample.

ii. Fourier Transform Infrared Spectroscopy

a. All IR analysis to determine the presence of the salt or freebase form of cocaine must be specifically requested and approved by the Laboratory Supervisor.
b. Place a small amount (1mg) of sample on to the crystal of the top plate and use the pressure arm to apply force to the sample.
c. Record the spectrum from 600 to 4000cm-1 and compare the peak spectra with a cocaine hydrochloride or cocaine base standard.
d. Document the results on the Drug Analysis Form and Control Card.

Criteria for Fourier Transform Infrared Spectroscopy

a. Library search match must be > 80%.
b. There must be a visual spectral match between the reference standard and the sample.***
c. At least 5 of the major wavelengths must be present for the sample.

F. Quantitation

i. All quantitative analysis must be specifically requested and approved by the Laboratory Supervisor.
ii. Prepare the standards as indicated in the reagent/standard preparation section. If the standards are already prepared, they must be at ambient temperature prior to use.
iii. Pipette out 0.8mL of each standard and place into individually labeled residue vial.
iv. Initiate autosampler sequence using the COCAINEQUANT method running chloroform blank solvent between each reference standard/s.
v. Check the concentration of each standard to determine if it meets the criteria of the laboratory.

vi. If standards are acceptable, continue with the analysis. If any of the standards are out of specification, notify the Laboratory Supervisor.
vii. For the sample: Dissolve 25mg of sample in 1mL of TPE stock solution and then bring to volume with chloroform using a 25mL volumetric flask. Mix the solution until dissolved.
viii. Pipette 2mL of the sample into a labeled GC vial and cap tightly. Prepare 2 separate GC vials for analysis.
ix. Initiate auto sampler sequence using the COCAINEQUANT method running chloroform blank solvent between each reference standard/s.
x. Sequence order should be similar: Blank, 1.0 standard, blank, 0.8 standard, blank, sample-1, blank, sample-2, blank, 0.4 standard, blank, 0.24 standard, blank, 1.0 standard and blank.
xi. Document the results on the Quantitation Analysis Form, Drug Analysis Form and Control Card.

Criteria for Quantitation

i. The concentration of each standard must be within +/- 2%. ****
ii. Results for the sample: take the average of both numerical results. If the sample is positive for cocaine base: results must be multiplied by a factor of 0.8929.
iii. If the average of the sample is greater than 100%, report as > 99%.

11. Documentation

A. All results will be documented on the Drug Analysis Form.
B. All raw data will be generated and filed according to the laboratory policy.
C. A certificate of analysis will be generated for each lab number which will document the results.

12. Attachments

GC Method

GC/MS Method